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Enantioselective determination of the novel antidepressant mirtazapine and its active demethylated metabolite in human plasma by means of capillary electrophoresis

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Abstract

Mirtazapine is a recent noradrenergic and specific serotonergic antidepressant drug. A capillary electrophoretic method has been developed for the enantioseparation and analysis of mirtazapine and its main active metabolite, *N*-desmethylmirtazapine, in human plasma. For method optimisation several experimental parameters were investigated, such as type and concentration of the chiral selector, buffer pH and capillary temperature. Baseline enantioseparation of the analytes was achieved in 2.5 min in a fused silica capillary (50 μm i.d.; 48.5 cm total length; 8.5 cm effective length) using carboxymethyl-β-cyclodextrin, dissolved in a background electrolyte consisting of 50 mM phosphate buffer at pH 2.5, as the chiral selector. UV detection was set at 205 nm. A careful pre-treatment of plasma samples was developed, using solid-phase extraction with hydrophilic–lipophilic balance cartridges (60 mg, 3 mL), eluting the sample with methanol, then concentrating it 37.5 times before injection. Extraction yield values are very satisfactory, being the average 89% for mirtazapine and 73% for *N*-desmethylmirtazapine. Application of the method to some human plasma samples has given satisfactory results.

Keywords: Enantiomer separation; Mirtazapine; Desmethylmirtazapine; Antidepressants

1. Introduction

Mirtazapine (1,2,3,4,10,14b-hexahydro-2-methyl-pyrazino[2,1-a]-pyrido[2,3-c][2-benzazepine], MRT, Fig. 1a), is a recent antidepressant, approved for the treatment of depression in June 1997. Combined with psychotherapeutic treatment, MRT has also recently been used to treat social anxiety during alcohol detoxification [1] and post-traumatic stress disorder [2].

MRT belongs to the class of the 'NaSSA' (noradrenergic and specific serotonergic antidepressant) drugs. In particular, MRT shows antagonism at α_2-adrenergic, 5-HT_2, 5-HT_3 and H_1 receptors, while anticholinergic and reuptake inhibition activities are almost absent [3].

MRT is considered to be quite effective in the treatment of depression [4], and seems to have an earlier onset of action when compared to both SSRI (selective serotonin reuptake inhibitor) and tricyclic antidepressants [5]. Daily doses are normally in the 15–60 mg range [6], supplied as film-coated tablets of Remeron containing 15 or 30 mg of active principle, and the resulting plasma levels are usually in the 30–80 ng/mL range [7]. MRT has a mean half-life of more than 20 h [8] and is extensively metabolised by hepatic cytochrome P450 enzymes (CYP), mainly CYP2D6 and CYP3A4 [9]; its two major metabolites are *N*-desmethylmirtazapine (DMR, Fig. 1b) and 8-hydroxymirtazapine. Of these, only DMR seems to possess pharmacological activity; it contributes 3–6% to the total pharmacodynamic profile of mirtazapine [10], and has a mean half-life of about 25 h [9].

Mirtazapine seems to be notably safe in overdose cases: plasma levels up to 2300 ng/mL (i.e. about 30 times the

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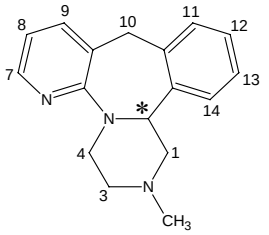
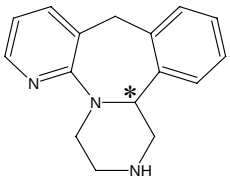
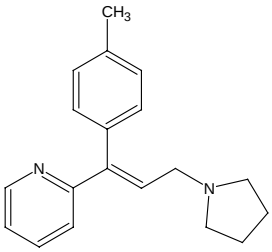


(a) MIRTAZAPINE (MRT)



(b) *N*-DESMETHYLMIRTAZAPINE (DMR)



(c) TRIPROLIDINE (I.S.)

Fig. 1. Chemical structures of the analytes (the asterisk indicates the chiral carbon atom) and the I.S.

therapeutic levels) have been observed after the intake of 30–50 times the prescribed daily dose, and the patients recovered without severe sequelae [11]. The main side effect of MRT is weight gain [12], although sedation, headache, dizziness and jaundice have also been reported [13].

MRT is a chiral compound, however it is administered clinically as a racemic mixture of *S*(+)- and *R*(−)-enantiomers. The two enantiomers differ in both pharmacokinetic and pharmacodynamic parameters [10]: in fact, e.g., the (+)-enantiomer is the more potent α-adrenoceptor antagonist of the two [14], however it reaches lower plasma levels and has a shorter half-life than its antipode [9]. Furthermore, the enantiomers seem to be metabolised by different CYP 450 isozymes: (+)-MRT is mainly metabolised by CYP2D6, while CYP1A2 and CYP3A4 show low metabolic activity towards (+)-MRT and (−)-MRT, respectively [15]. Thus, a chiral separation of the enantiomers may be important in pharmacokinetic studies and for evaluating the clinical response of patients during therapeutic drug monitoring (TDM). Nowadays, TDM is considered a powerful tool for therapy optimisation and personalisation [16–19], and is a constantly expanding practice in psychiatric clinics, mainly for antipsychotic agents; in the last few years, however, this practice has also been advised for antidepressants [17,20], especially when non-compliance or metabolic anomalies are suspected, or during polypharmacy [21]. This of course requires suitable and feasible analytical methods for the reliable determination of psychotropic drugs and their active metabolites in biological fluids.

Some methods are reported in the literature for the analysis of MRT in human plasma [10,22–26] using liquid chromatography, with spectrophotometric [23] or spectrofluorimetric detection [10,22,24,26], or gas chromatography–mass spectrometry [25]. A few of these methods determine DMR as well [23,26], however only one of them carries out a chiral determination of MRT [24]. To our best knowledge, until now no capillary electrophoretic (CE) method has been developed for the analysis of MRT in plasma, although two CE methods do exist for MRT analysis in formulations [27,28]. Furthermore, no method, electrophoretic or otherwise, carries out the simultaneous determination of MRT and DMR enantiomers. Thus, the aim of this study is the development of a fast and feasible CE method for the enantioseparation of MRT and its active metabolite, and its application to human plasma samples for toxicological and therapeutic monitoring purposes. Due to its characteristics of high selectivity, efficiency and velocity, and the use of minute amounts of chiral selectors, CE is particularly suitable for the enantioseparation of chiral compounds [29–34]. The main disadvantage of CE is its limited sensitivity, which can, however, be compensated by a suitable pre-concentration of MRT and DMR present in the biological samples.

In this paper, several parameters, such as chiral selector, buffer pH and capillary temperature were studied for enantioseparation optimisation, by means of capillary zone electrophoresis. An original plasma sample pre-treatment and pre-concentration procedure by solid-phase extraction (SPE) was implemented; the method was then validated in terms of extraction yield and precision, and applied to plasma samples from depressed patients treated with Remeron tablets.

2. Experimental

2.1. Chemicals and solutions

Mirtazapine maleate and *N*-desmethylmirtazapine pure compounds were kindly provided by Organon (Oss, The Netherlands).

All chemicals were analytical grade or better; β-cyclodextrin (β-CD); carboxymethyl-β-cyclodextrin (CM-β-CD); β-CD sulphate sodium salt and γ-CD were purchased from Fluka (Buchs, Switzerland); sulfobutyl ether-β-CD was from CyDex (Overland Park, KS, USA). Boric acid, 85% (w/w) phosphoric acid, citric acid, glacial acetic acid, methanol, acetonitrile, 2 M sodium hydroxide and 37% (w/w) hydrochloric acid were from Carlo Erba (Milan, Italy). Triprolidine used as the internal standard (I.S., Fig. 1c) was purchased from Sigma (St. Louis, MO, USA).

Ultrapure water (18.2 MΩ cm) was obtained by means of a Millipore (Bedford, MA, USA) Milli-Q apparatus.

Stock solutions of the analytes (1 mg/mL) were prepared by dissolving suitable amounts of the pure substance in methanol. Standard solutions were obtained by diluting stock solutions with a methanol–pH 2.5, 5 mM phosphate buffer (30:70, v/v) mixture. Stock solutions were stable for at least 2 months when stored at −20 °C (as assessed by

spectrophotometry); standard solutions were prepared fresh every day.

The background electrolyte (BGE) was a pH 2.5, 50 mM phosphate buffer containing 0.24% (w/v) CM-β-CD, prepared as follows: 340 μL of 85% phosphoric acid were dissolved in about 80 mL of water and the solution was brought to pH 2.5 with 2 M NaOH. The solution was then transferred into a 100 mL volumetric flask and diluted to the mark. To 2 mL of this buffer, 4.8 mg of CM-β-CD were added and the mixture sonicated for 5 min and filtered through a cellulose acetate syringe filter (0.20 μm, Albet-Jacs, Barcelona, Spain) prior to use.

2.2. Apparatus and electrophoretic conditions

All assays were carried out on an Agilent (Waldbronn, Germany) ^{3D}CE apparatus equipped with a diode array UV detector. The analyte separation was carried out on an uncoated fused silica capillary [Composite Metal Services, Hallow, UK; 48.5 cm (8.5 cm effective length) × 50 μm i.d. × 375 μm o.d.]. Peak detection was done at 205 nm.

A constant voltage of −20 kV was applied and the sample was injected by pressure (50 mbar, 20 s) at the anodic end of the capillary. The capillary was thermostatted at 12.5 °C. Preliminary assays were carried out using an effective capillary length of 40.0 cm and applying a voltage of +20 kV.

Before use, new capillaries were washed with water (10 min), 1 M NaOH (10 min) and water again (20 min), then conditioned with the BGE for 15 min before injecting (the 15 min conditioning was repeated each time the BGE composition was changed). Before each electrophoretic run, the capillary was conditioned with the BGE for 2 min; after the electrophoretic run it was sequentially rinsed at high pressure (6 bar) with: water (30 s), 0.1 M HCl (30 s), water (30 s), 0.1 M NaOH (30 s) and water again (60 s). For storage overnight, the capillary was rinsed with water for 5 min, 0.1 M NaOH for 5 min and water again for 10 min.

2.3. Human plasma sampling

"Blank" plasma samples were obtained from healthy volunteers not subjected to any pharmacological therapy. The samples were drawn into test tubes containing EDTA as an anticoagulant and centrifuged at 1400 × *g* for 15 min. The supernatant plasma was transferred into test tubes and frozen at −20 °C until analysis. This procedure was also used to separate plasma from the blood of depressed patients of the Mental Health Department (AUSL Bologna), who were undergoing therapy with Remeron.

2.4. Sample pretreatment: SPE procedure

For the SPE procedure Oasis HLB (hydrophilic–lipophilic balance) cartridges (60 mg, 3 mL) from Waters (Milford, MA, USA) were used. The sorbent of these cartridges is a macroporous polymer made from two monomers, the lipophilic divinylbenzene and the hydrophilic *N*-vinylpyrrolidone.

The cartridges were activated with 3 mL of methanol twice and conditioned with 3 mL of water twice, then loaded with 1.5 mL of plasma diluted with 1.5 mL of water and spiked with 50 μL of triprolidine (I.S.) solution at the concentration of 1.5 μg/mL (patient's plasma) or analyte and I.S. standard solution (blank plasma).

The cartridges were washed with 1 mL of water, 2 × 1 mL of a methanol–pH 9.0, 50 mM borate buffer (60:40, v/v) mixture, 1 mL of water and finally 150 μL of methanol. The analytes were then eluted with 1.0 mL of methanol. The eluate was brought to dryness in a rotary evaporator and redissolved with 40 μL of a methanol–pH 2.5, 5.0 mM phosphate buffer (30:70, v/v) mixture. The resulting solution was injected into the capillary electrophoresis apparatus.

2.5. Method validation

2.5.1. Calibration curves

Six-point calibration curves in plasma were set up for each racemic analyte in the 15.0–300.0 ng/mL concentration range (corresponding to 7.5–150.0 ng/mL of each single enantiomer), by adding 50 μL of a suitable standard solution to 1.50 mL of blank plasma and subjecting the resulting mixture to the described SPE procedure. After CE analysis, the analyte/I.S. peak area ratio (a pure number) was plotted as a function of the added concentration (expressed as ng/mL), and the calibration curves were obtained by means of the least-square method.

2.5.2. Precision assays

Spiked plasma samples were prepared and analysed six times within the same day to obtain repeatability values and six times over different days to obtain intermediate precision values, according to USP XXVI requirements [35]. Each assay was carried out at three different levels (racemic concentration: 30, 150 and 300 ng/mL for MRT; 20, 70 and 150 ng/mL for DMR) and on a different plasma sample. The quantitation limit (LOQ) was calculated with the formula $10\sigma S^{-1}$ (where σ is the standard deviation of the response and S is the slope of the calibration curve) and the detection limit (LOD) was obtained as the concentration whose signal/noise ratio is 3. Both procedures are in accordance to USP XXVI [35] and Crystal City [36] guidelines.

3. Results and discussion

3.1. Enantioseparation of mirtazapine and N-desmethylmirtazapine

Preliminary spectrophotometric assays confirmed that MRT and DMR have very similar absorbance spectra, with two relative maxima at 205 and 220 nm; the maximum at

220 nm, however, is much lower than that at 205 nm. For this reason, it was decided to carry out analyte detection at the wavelength of 205 nm, which confers higher sensitivity.

MRT is a basic compound ($pK_a = 8.1$[1]), and as such can be ionised at acidic pH values. For this reason, its electrophoretic behaviour was investigated starting from the previous experience of the authors in the field of electromigration separation of basic drugs such as fluoxetine, citalopram and other SSRI drugs [31,37–39].

An acidic (phosphate) buffer containing a negatively charged cyclodextrin was thus judged as the most suitable for this enantioseparation. Preliminary studies with different buffers at pH values ranging from 2.5 to 6.0 have demonstrated that MRT and DMR can be baseline separated using a simple pH 2.5, 50 mM phosphate buffer and an effective capillary length of 40.0 cm, operating at 25 °C. Keeping this buffer as the basis for the BGE, different charged or chargeable cyclodextrins (namely CM-β-CD, β-CD sulphate and sulfobutyl ether-β-CD) were added to obtain the chiral separation of the analytes; two native cyclodextrins (β-CD and γ-CD) were also tried in order to obtain more comprehensive data. The use of native cyclodextrins originated only weak interaction with the analytes (small increase of migration times); thus no enantioseparation was reached. On the contrary, the two permanently charged CDs (β-cyclodextrin sulphate and sulfobutylether-β-cyclodextrin) interacted very strongly with the analytes even at very low concentrations (0.01%, w/v), leading to peak asymmetry and loss of efficiency. Best results were obtained using CM-β-CD, which is expected to have a very low percentage of ionisation at pH 2.5.

Baseline separation of each enantiomeric couple (within pair) was obtained using rather low concentrations of CM-β-CD (0.20–0.40%, w/v), however a degree of peak overlapping occurred between the two analytes (between-pair), due to their closeness. For this reason, it was decided to lower the operating temperature, which usually reduces the mobility of the compounds. Temperature was studied in the 10–25 °C range; the result was that peak resolution improved when lowering the temperature, at the cost of longer run times. Best results were obtained at 12.5 °C, with baseline separation of all enantiomers and acceptable run times (<12 min). An example of a standard solution analysed with a BGE composed of pH 2.5, 50 mM phosphate buffer containing 0.25% CM-β-CD, injecting the sample at the anodic end of the capillary (effective length: 40.0 cm) and applying a voltage of +20 kV, is reported in Fig. 2.

In order to obtain shorter run times, a reduced effective length of the capillary (8.5 cm) was used, injecting the samples at the short end of the capillary and reversing the polarity of applied voltage. With this method run times are notably shorter (less than 3 min instead of 12) while

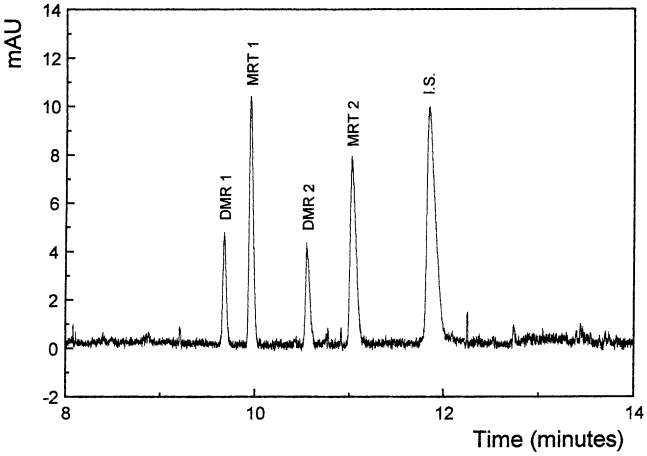


Fig. 2. Electropherogram of a standard solution containing 5.0 μg/mL of racemic MRT, 2.5 μg/mL of racemic DMR and 5.0 μg/mL of the I.S. (triprolidine). Electrophoretic conditions: uncoated fused silica capillary [48.5 cm (40.0 cm effective length) × 50 μm i.d. × 375 μm o.d.]; BGE: pH 2.5, 50 mM phosphate buffer containing 0.24% (w/v) CM-β-CD; injection: pressure (50 mbar, 20 s) at the anodic end of the capillary; applied voltage: 20 kV; detection wavelength: 205 nm; capillary temperature: 12.5 °C.

the baseline separation of the analytes is maintained. As an example of the good enantioresolution of the method, the electropherogram of a standard solution containing 5.0 μg/mL of MRT, 2.5 μg/mL of DMR and 5.0 μg/mL of the I.S. (triprolidine) is reported in Fig. 3. As can be seen, all the peaks are fully resolved, with the following migration times: DMR 1, 1.68 min; MRT 1, 1.76 min; DMR 2, 1.85 min; MRT 2, 2.01 min; I.S., 2.33 min.

3.2. Sample pre-treatment

Sample pre-treatment is a critical step in any biological sample analysis, and it becomes even more crucial in this case, since CE lacks the sensitivity necessary to reliably

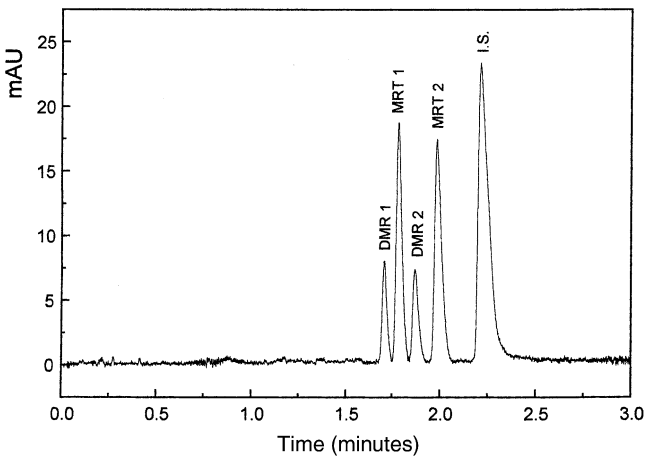


Fig. 3. Electropherogram of a standard solution containing 5.0 μg/mL of racemic MRT, 2.5 μg/mL of racemic DMR and 5.0 μg/mL of the I.S. (triprolidine). Electrophoretic conditions: as in Fig. 2, except effective capillary length: 8.5 cm; applied voltage: −20 kV.

[1] Calculated using Advanced Chemistry Development (ACD) Software Solaris V4.67 (© 1994–2004 ACD).

determine the low levels of MRT and its metabolite usually found in patient plasma. For this reason, both purification and concentration of the biological samples were accomplished with a sample pre-treatment step carried out by SPE. High-capacity (60 mg, 3 mL) Oasis HLB cartridges were chosen for this purpose: they can be loaded with high plasma volumes (1.5 mL) while keeping optimal analyte retention. In fact, from preliminary assays it was found that no amount of the analytes was lost during cartridge loading.

The washing procedure was chosen after several preliminary assays. First of all, it was decided that a mixture of basic buffer and methanol would be used for this step, in order to avoid analyte protonation while washing out as much matrix components as possible. Thus, a pH 9.0 borate buffer mixed with increasing amounts (10–90%) of methanol was used for these assays. It was observed that the less lipophilic analyte, DMR, is eluted with methanol percentages higher than 70%; for this reason, a pH 9.0 borate buffer–methanol (40:60, v/v) mixture was chosen as the best compromise between sample purification and analyte recovery.

Then, the analytes were eluted with 1 mL of methanol, which was then dried under vacuum and redissolved with 40 μL of a phosphate buffer–methanol mixture (thus obtaining a 37.5-times concentration with respect to the original sample). When the resulting solution was analysed by CE, good purification and extraction yield values were obtained for all analytes, however strong interference was observed on the I.S. peak. Thus, the SPE procedure was modified to achieve better purification. It was observed that the first 150 μL of methanolic eluate did not contain significant amounts of the analytes and could therefore be discarded. This step was thus added to the procedure as a last washing step, to be performed before the final elution with 1 mL of methanol.

The electropherogram of a plasma sample from a healthy volunteer spiked with 50 ng/mL of MRT and 30 ng/mL of DMR, after SPE and CE analysis, is shown in Fig. 4a. For comparison, the electropherogram of a blank plasma sample without analyte spiking is reported in Fig. 4b. As can be observed, the analyte separation is comparable to that obtained on standard solutions, and the plasma matrix does not interfere with either the analyte peaks or the I.S. peak.

It should be noted that the plasma pre-treatment procedure is so efficient that up to 3.0 mL of plasma can be loaded onto the cartridge, and the resulting electropherogram is still devoid of interference and absolute recovery values are higher than 80%. In this case the analytes are concentrated 75 times with respect to the original plasma sample. Therefore, this latter procedure can be used to quantify lower levels of the analytes, at the cost of taking higher blood volumes from the patients.

3.3. Method validation

The developed procedure was therefore applied to spiked blank plasma samples for validation purposes. Six-

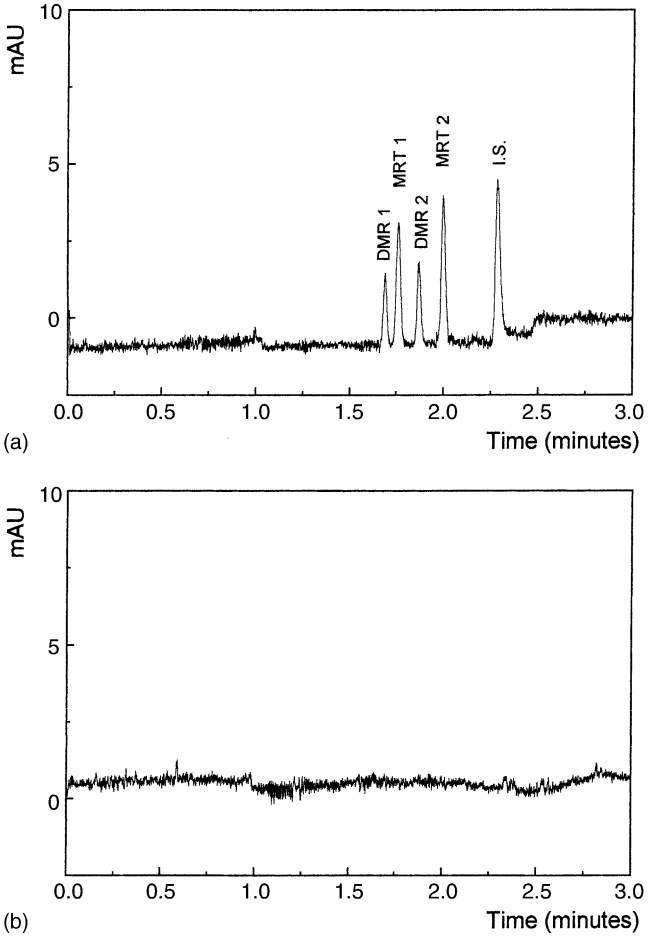


Fig. 4. Electropherograms of (a) a blank plasma sample spiked with 50 ng/mL of racemic MRT, 30 ng/mL of racemic DMR and 50 ng/mL of the I.S. and then subjected to the SPE procedure and (b) the same blank plasma sample, without any spiking, after the SPE procedure. Electrophoretic conditions: as in Fig. 3.

point calibration curves were set up in the 15–300 ng/mL concentration range (racemic concentrations), obtaining good linearity (Table 1). Since a 37.5-times sample concentration is introduced with the SPE procedure, and since the LOQ on standard solutions was 400 ng/mL (racemic concentration), a satisfactory LOQ on plasma samples corresponding to 5 ng/mL of each enantiomer is reached. The limit of detection (LOD) on standard solutions was 200 ng/mL, and consequently the LOD on plasma samples resulted to be 3 ng/mL for each enantiomer.

Extraction yield (absolute recovery) assays were carried out at three different levels of sample spiking, and gave good results (Table 2): extraction yield values higher than 87% were obtained for both MRT enantiomers, and higher than 73% for DMR enantiomers. The mean recovery of the I.S. is 86%. Reproducibility data are also reported in Table 2: as one can see, these results are satisfactory as well.

Table 1
Linearity parameters

Analyte	Linearity equation ($y = a + bx$)[a]		Correlation coefficient, r_c	Standard deviation	
	a	b		a	b
MRT 1	−0.071	1.384	0.9981	0.057	0.012
MRT 2	−0.071	1.437	0.9977	0.070	0.013
DMR 1	−0.067	1.001	0.9965	0.058	0.009
DMR 2	−0.065	1.072	0.9959	0.056	0.009

[a] y is the analyte/I.S. peak area ratio, expressed as a pure number; x is the analyte concentration, expressed as μg/mL.

Table 2
Extraction yield and precision data[a]

Analyte	Concentration (ng/mL)	Extraction yield (%)	Repeatability (R.S.D., %)	Intermediate precision (R.S.D., %)
MRT 1	15	88	5.3	5.6
	75	88	1.9	2.0
	150	92	1.0	1.2
MRT 2	15	88	5.3	5.5
	75	87	1.9	2.1
	150	93	0.9	1.2
DMR 1	10	73	6.3	6.5
	35	74	2.5	3.2
	75	74	2.0	2.2
DMR 2	10	73	6.1	6.2
	35	73	2.5	3.3
	75	73	2.1	2.3
I.S.	50	86	2.4	2.6

[a] All values were obtained from six different assays.

3.4. Selectivity

Selectivity assays were carried out by injecting standard solutions containing different drugs often administered during psychiatric therapy: a complete list can be found in Table 3. As can be seen, none the drugs tested interfered with the determination of MRT and DMR enantiomers, except olanzapine. It was found, however, that olanzapine can be eliminated during the SPE procedure if a third millilitre of borate buffer–methanol mixture is added to the washing step.

Table 3
Drugs tested as possible interference

Compound	Migration time (min)
Antidepressant drugs	
Imipramine	n.d.
Dibenzepine	3.5
Protriptiline	n.d.
Antipsychotic drugs	
Chlorpromazine	n.d.
Clozapine	3.7
Desmethylclozapine	2.9
Olanzapine	1.7
Other drugs	
Prometazine	n.d.
Melatonin	n.d.
Paracetamol	n.d.
Acetylsalycilic acid	n.d.

n.d.: not detected within a 7-min run.

3.5. Application to patient plasma samples

Having thus validated the method, it was applied to plasma samples taken from patients of the Mental Health Department (AUSL Bologna) who were treated with Remeron tablets. A plasma sample from a patient treated with 30 mg/day of MRT, after SPE and CE analysis is reported in Fig. 5. The electropherogram is completely devoid of interfering peaks, and the separation of the analytes is still good. The analyte levels found in this sample were 17 ng/mL for MRT 1; 9 ng/mL for MRT 2; 10 ng/mL for DMR 1; 7 ng/mL for DMR 2. In this and all other samples analysed by us, the concentration of MRT 1 found is always higher than that of MRT 2, and the concentration of DMR 1 is higher than that of DMR 2. It can thus be supposed that MRT 1 corresponds to *R*(−)-MRT, while MRT 2 corresponds to *S*(+)-MRT. In fact, the available enantioselective pharmacokinetic data [9] points out to the fact that *R*(−)-MRT reaches higher plasma levels than *S*(+)-MRT when racemic MRT is administered. Analogously, DMR 1 could be *R*(−)-DMR and DMR 2 could be *S*(+)-DMR: of course, circular dicroism studies or the injection of pure enantiomers would be necessary to confirm these tentative identifications.

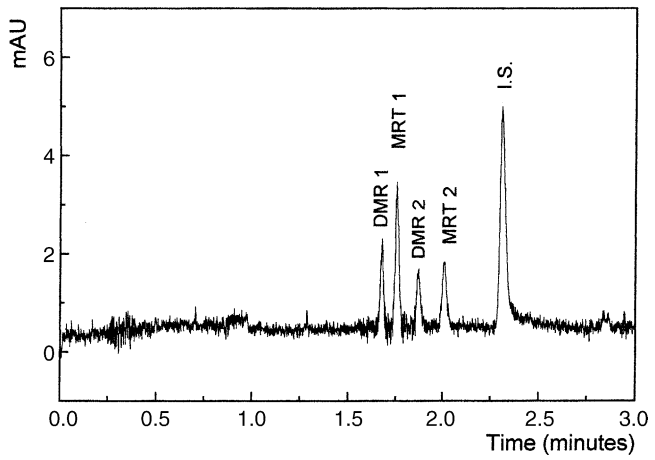


Fig. 5. Electropherogram of a plasma sample form a patient treated with 30 mg/day of MRT, after the SPE procedure. Electrophoretic conditions: as in Fig. 3.

Accuracy, assessed by means of recovery assays, resulted to be good. In fact, when plasma samples from patients treated with Remeron were spiked with 30 ng/mL of MRT and 20 ng/mL of DMR, mean recovery values of 90% for MRT and 75% for DMR were obtained.

4. Conclusion

The method presented herein, based on capillary zone electrophoresis with carboxymethyl-β-cyclodextrin as the chiral selector, allows to enantioseparate MRT and its main metabolite DMR within a very brief time (less than 3 min). The method was applied to some plasma samples from depressed patients, using a careful SPE procedure for biological sample pre-treatment. Besides a notable sample cleaning from matrix compounds, this pre-treatment allows to concentrate the samples 37.5 times, thus obtaining the sensitivity needed to reliably determine the low plasma levels found during therapy.

The method has been validated and has given good results in terms of linearity, precision (with R.S.D. values lower than 6.5%), and extraction yield (mean: 89% for MRT, 73% for DMR).

The method is thus suitable for the determination of MRT and DMR, both at therapeutic and toxic levels, in plasma of patients treated with Remeron tablets.

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